Maternal effects on offspring fitness in *Pseudemoia entrecasteauxii*: selective advantages and physiological mechanisms

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

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Preface

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text of this thesis.

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Statement of Co-Authorship

Publications produced as part of this thesis

Chapter 2. Itonaga K, Wapstra E and Jones SM. A novel pattern of placental leucine transfer during late pregnancy in a highly placentotrophic viviparous lizard. *Journal of Experimental Zoology Part B Molecular & Developmental Evolution*. [in review]
Contribution: Itonaga 75%, Wapstra 10% & Jones 15%

Chapter 3. Itonaga K, Wapstra E and Jones SM. Evidence for placental transfer of maternal corticosterone in a viviparous lizard. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*. [in press]
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Chapter 4. Itonaga K, Jones SM. and Wapstra E. Effects of variation in maternal basking and food quantity during gestation provide evidence for the selective advantage of matrotrophy in a viviparous lizard. *Oecologia*. [to submit]
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Chapter 6. Itonaga K, Jones SM. and Wapstra E. Maternal effects in a viviparous lizard: effects of variation in maternal carotenoids intake during gestation on offspring immune response. *Functional Ecology*. [in press]
Contribution: Itonaga 75%, Jones 10 % & Wapstra 15%

Abstract

This thesis focused on why matrotrophy has evolved in viviparous (live-bearing) reptiles. Matrotrophic reproduction is direct supply of nutrients by the mother during gestation (e.g. placental support), and it is rare in viviparous reptiles. Although a large number of studies have investigated the evolution of viviparity in reptiles, we know comparatively little about the evolution of matrotrophic viviparity in reptiles. Matrotrophic reproduction implies complex and increasing maternal-embryonic communications such as nutrient and hormone transfer via the placenta during gestation. These placental nutrient and hormone transfers affect offspring phenotype and, therefore, fitness. Such non-genetic effects on offspring phenotype are so-called maternal effects. However, the importance of maternal effects is still the subject of an ongoing debate in terms of fitness. In addition, there is a very little information on maternal effects and their relation to physiological mechanisms in viviparous reptiles, especially matrotrophic viviparous reptiles.

Pseudemoia entrecasteauxii is one of the few known species of matrotrophic viviparous reptiles. The first section of this thesis focused on physiological mechanisms during gestation in *P. entrecasteauxii* to explore how the timing of placental nutrient and hormone transfer during embryogenesis affects embryonic development and consequently offspring fitness. The second section of this thesis focused on maternal effects and their adaptive significance in *P. entrecasteauxii*. In matrotrophic viviparous fish, for example, the evolution of matrotrophy may have been related to high maternal energy availability during gestation, which enhances offspring fitness through maternal effects. In reptiles, net energy gain is dependent on the interaction between body temperature, plasma corticosterone concentration and food availability. The effect of maternal net energy gain during gestation on offspring phenotype was examined in two experiments. In the first, the effect of variation in maternal thermal condition and maternal food availability during gestation was investigated. In the second, investigation of the effect of variation in maternal plasma corticosterone concentration and maternal food availability during gestation was conducted. For each experiment, offspring growth rate was examined using reciprocal transplant experiments (i.e. investigation of adaptive significance of maternal effects) because offspring growth rate is usually associated with fitness. In the final experiment, the effects of maternal food quality (i.e. food with β-carotene and food without β-carotene) during gestation on offspring phenotype were investigated by

measuring offspring immune capacity in response to antigenic stimulation. I found that the degree of maternal nutrient support during gestation significantly influenced offspring phenotype, and high maternal net energy availability during gestation potentially enhanced offspring fitness. These findings suggest that predictably high maternal energy availability during gestation may have been an important determinant for the evolution of matrotrophic viviparity in *P. entrecasteauxii*. Furthermore, findings on the effects of maternal corticosterone and maternal β-carotene availability during gestation on offspring phenotype suggest several important considerations for offspring fitness as a consequence of the evolution of matrotrophic reproduction in *P. entrecasteauxii*. Thus, this thesis contributes significantly to our understanding of the evolution of matrotrophic viviparity in reptiles and also indicates a further direction for research into this topic.

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Chapter 1. General introduction

Amongst vertebrates, viviparity (live-bearing) has evolved in fish, amphibians, reptiles and mammals, but not in birds (Blackburn, 1981; Wourms, 1981; Shine, 1985; Blackburn & Evans, 1986; Blackburn, 1992; Wourms & Lombardi, 1992; Duellman & Trueb, 1994). Furthermore, it has evolved in at least 130 independent lineages, with about 100 of these lineages in reptiles (Shine, 1985; Blackburn, 1992). These multiple origins suggest pervasive benefits to viviparity across a wide range of taxa, life histories and habitats (Roff, 1992; Andrews & Mathies, 2000). However, there may be costs associated with viviparity; the biggest costs are usually regarded as a reduced number of reproductive opportunities, reduced clutch size, increase in energy cost and predation (Fitch, 1970; Tinkle & Gibbons, 1977; Shine, 1980; Vitt & Blackburn, 1983, 1991; Vitt, 1986; Heatwole & Taylor, 1987; Adolph & Porter, 1993; Hutchinson et al., 2001; Wapstra & O'Reilly, 2001).

1-1. Evolution of viviparity in reptiles

In reptiles, viviparity is widespread amongst squamate reptiles (lizards and snakes). It occurs in about 20 % of extant reptiles (Blackburn, 1982, 1992; Shine, 1985). However, viviparity does not occur in turtles/tortoise, crocodilians, or tuatara (Blackburn, 1982; Shine, 1985). Squamate reptiles are especially suited to the study of the evolution of viviparity because they show a greater number of independent evolutionary events leading to viviparity than other vertebrate species (Blackburn, 1992). Furthermore, selective forces in the evolution of viviparity in reptiles may be similar to other viviparous vertebrate species (Tinkle & Gibbons, 1977; Shine, 1983, 1995; Wourms & Lombardi, 1992; Wake, 1993). If this is so, studies of the evolution of viviparity in reptiles may contribute to our understanding of the evolution of viviparity in other vertebrate species. In contrast, mammalian species cannot be used as models to understand viviparity because it has evolved from one single event in that clade.

The selective factors related to the evolution of viviparity in reptiles have been considered since the early twentieth century (e.g. Weekes, 1935; Neill, 1964; Packard, 1966; Greene, 1970; Packard et al., 1977; Shine & Berry, 1978; Shine & Bull, 1979; Shine, 1983, 1995; Qualls & Andrews, 1999; Hodges, 2004), and a number of hypotheses proposed. For example, the "dry or hot environment hypothesis" focuses on egg desiccation (Weekes, 1935). The "aquatic ancestor hypothesis" focuses on the limitation of suitable nest sites in aquatic habitats (Neill, 1964) or on the evolution of egg formation from aquatic

environments to terrestrial environments (Packard, 1966) and the "parental care hypothesis" focuses on nest guarding (Neill, 1964; Shine & Bull, 1979). However, since the 1980s, the predominant hypothesis for the evolution of viviparity in reptiles has been the "cold climate hypothesis" (Tinkle & Gibbons, 1977; Shine, 1983) and its derivatives. This hypothesis focuses on the advantages of thermal benefits for embryonic development in cold areas: maternal thermoregulation promotes more rapid embryonic development and enhances egg survivorship compared with a cold nest (Tinkle & Gibbons, 1977; Shine, 1983).

Shine (1995) further developed the cold climate hypothesis (Shine, 1983) because it did not explain why some viviparous reptiles have evolved in tropical climates. He suggested that prolonged uterine retention may enhance offspring fitness because eggs incubated at maternal body temperature may produce offspring with higher fitness due to changes in morphology, physiology and behaviour) than do eggs incubated at normal nest temperature. These non-genetic effects on offspring phenotype are called "maternal effects" (Mousseau & Fox, 1998) (discussed in Section 1-4). Indeed, temperature during embryogenesis affects fitness related offspring phenotypic traits in viviparous reptiles (Schwarzkopf & Shine, 1991; Van Damme et al., 1992; Swain & Jones 2000; Wapstra, 2000; Wapstra et al., 2009, 2010; While et al., 2009), and embryonic temperature is controlled by maternal thermoregulation (reviewed in Robert & Thompson, 2010). Thus, it is possible that prolonged uterine retention of eggs may enhance offspring fitness through maternal control (e.g. maternal thermoregulation during gestation). This idea is called the "Maternal Manipulation Hypothesis". Further studies (e.g. Qualls, 1997; Webb et al., 2006; Ji et al., 2007) have demonstrated that maternal thermoregulation during gestation may provide selective advantages in a wide range of climate contexts in viviparous reptiles. Currently, therefore, this hypothesis is more generally accepted, but an ongoing debate remains because of the uncertain benefits of maternal effects (discussed in Section 2).

1-2. Evolutionary steps in the evolution of viviparity in reptiles

The evolutionary transition from oviparity to viviparity in reptiles required several successive changes: endocrine control systems (Shine & Guillette, 1988; Guillette, 1993), reduction of eggshell thickness (Packard et al., 1977; Guillette, 1993); and evolution of maternal-embryonic connections (Weekes, 1935; Stewart & Thompson, 2000). The

development of endocrine control (i.e. hormone secretion) allows prolonged uterine retention of eggs. For example, after ovulation, egg retention in some reptilian species is maintained by progesterone (Guillette et al., 1991) which is secreted by organs such as the postovulatory follicles (corpora lutea) (Rothchild, 1981; Shine & Guillette, 1988). In some reptiles, oviposition takes place rapidly when maternal plasma progesterone concentrations decline (Shine & Guillette, 1988; Guillette et al., 1991; Cree et al., 1992). In some viviparous reptiles, maternal plasma progesterone concentrations remain high throughout pregnancy and markedly decrease close to delivery (Guillette, 1987; Xavier, 1987; Jones et al., 1997; also see Jones, 2010). Therefore, the ability to maintain progesterone secretion and keep high plasma progesterone concentrations during pregnancy is essential for prolonging uterine retention in some viviparous reptiles. Endocrine control may also support important maternal-embryonic communication (Guillette et al., 1991). For example, the role of corticosterone as a signal for the timing of parturition has been well documented in mammals (Liggins, 1969; Keller-Wood & Wood, 2001; Jenkin & Young, 2004). Corticosterone produced by embryos at the end of embryonic development may also act to determine the timing of parturition in viviparous reptiles (Painter et al., 2002; Girling & Jones, 2006).

Another requirement for the evolution of viviparity in reptiles is a reduction in eggshell thickness. The eggshell is a physical barrier to gaseous exchange by diffusion (Packard et al., 1977), but embryonic development requires oxygen especially during late embryogenesis (Guillette, 1982; Andrews, 2002; Shine & Thompson, 2006). The reduction of shell thickness decreases the distance between the mother and the embryos, enhancing gaseous diffusion. The reduction of eggshell thickness is thus an essential stage in the evolution of viviparity. For example, Qualls (1996) showed a positive relationship between the length of egg retention and loss of eggshell thickness in *Lerista bougainvillii*, a species that demonstrates variation in egg retention [also see the eggshell of *Zootoca vivipara* (Heulin, 1990)]. The development of the placentae (i.e. the maternal-embryonic connection) also facilitates gaseous exchange (Thompson et al., 2004). In addition, other functions of reptilian placentae are water exchange (Stewart & Thompson, 2000), hormone regulation (Guarino et al., 1998; Painter et al., 2002; Painter & Moore, 2005) and at least a small amount of inorganic nutrient (e.g. calcium, magnesium, sodium and potassium) and/or organic nutrient (e.g. amino acid & fatty acid) transfer (Blackburn,

1993; Swain & Jones, 1997; Thompson et al., 2002; Jones & Swain, 2006; Thompson & Speake, 2006; Stewart et al., 2009).

The degree of placental nutrient support (i.e. yolk and placenta dependency) is usually associated with the complexity of placental structure among viviparous reptiles (reviewed in Thompson et al., 2000, 2002). In viviparous reptiles, four types of placentae (i.e. Type I - IV placentae) have been described based on the structure of the chorioallantoic placenta (Weekes, 1935; Blackburn, 1993). Basically, Type I and Type II placentae have simple structures. The embryos of females with these placentae are usually nourished primarily by yolk laid down before gestation (i.e. predominantly lecithotrophy) (Stewart & Thompson, 2000; reviewed in Thompson et al., 2002). Conversely, Type III and Type IV placentae show complex structures. The females with these placentae may demonstrate significant placental contribution to embryonic nutrition during gestation with reduced reliance on the yolk (i.e. high levels of matrotrophy or placentotrophy) (Stewart & Thompson, 2000; Thompson et al., 2000; Thompson & Specks, 2006; reviewed in Thompson et al., 2002). In addition, Type III and IV placentae may develop a special region (placentome), for nutrient transfer (Blackburn, 1992; Stewart & Thompson, 1996; Ramírez-Pinilla et al., 2006). Each type of placenta may have evolved independently (Blackburn, 1992; Blackburn & Vitt, 2002). The majority of viviparous reptiles are predominantly lecithotrophic; high levels of matrotrophy occur in only a few taxa (Weekes, 1935; Blackburn, 1992, 2006; Thompson et al., 2002).

1-3. The costs and benefits of matrotrophy in viviparous reptiles

Matrotrophic species, which demonstrate direct nutrient support for embryonic development during gestation (e.g. placental nutrient support = placentotrophy), have evolved independently from a lecithotrophic ancestor (i.e. using yolk for embryonic development) with at least 24 independent origins in vertebrates. At least four of these origins are in reptiles (Blackburn, 1992, 1998, 2006; Stewart, 1992; Wake, 1992; Wourms & Lombardi, 1992; Hamlett & Hysell, 1998; Flemming & Branch 2001; Reynolds et al., 2002). A key difference in the reproductive strategy between lecithotrophy and matrotrophy is the timing of energy investment. In short, lecithotrophic reproduction invests energy before fertilisation, whereas matrotrophic reproduction invests energy after fertilisation. Therefore, lecithotrophic reproduction may have advantages in a fluctuating environment (i.e. food levels during embryonic development) because it packs all energy

for embryonic development before embryogenesis (Thibault & Schultz, 1978; Wourms & Lombardi, 1992).

The costs and benefits of matrotrophic reproduction may depend on the fluctuations of food levels during gestation (Trexler & DeAngelis, 2003). For example, with abundant food environments after fertilisation, matrotrophic females may maintain a large clutch size at fluctuations and/or invest more energy in current reproduction to enhance offspring fitness (Trexler & DeAngelis, 2003; Marsh-Matthews & Deaton, 2006; Ostrovsky et al., 2009), and this idea links to beneficial maternal effects (see next section). In contrast, if food availability reduces after fertilisation, a large clutch size may be costly (Trexler & DeAngelis, 2003). Therefore, some literature (e.g. Brooks & McLennan, 1991; Trexler & DeAngelis, 2003) has suggested the presence of pre-adaptations for matrotrophic reproduction such as the bet-hedging strategy (i.e. selective energy allocation among embryos), abortion of embryos and energy recycling which is already invested (Kozlowski & Stearns, 1989; Greeff et al., 1999; Trexler & DeAngelis, 2003; reviewed in Gaillard & Yoccoz, 2003). These strategies may regulate the maternal energy investment and maternal energy budget during gestation.

Most viviparous reptiles, strictly speaking, demonstrate at least some degree of matrotrophy or placentotrophy in which inorganic and/or organic nutrients are directly supplied by the mother during gestation (Thompson et al., 2000, 2002; Blackburn, 2006). Many viviparous reptiles display less than 1 % of placental support for embryonic development (Thompson et al., 2002). However, we currently know some viviparous reptiles (i.e. *C. chalcides*, *Eumecia anchietae*, *Mabuya bistriata, M. heathi, M. mabouya*, *Niveoscincus ocellatus*, *Pseudemoia entrecasteauxii*, *P. pagenstecheri* and *P. spenceri*) display high levels of matrotrophy (more than 22 % of embryonic development is supported by matrotrophy) (Ghiara et al., 1987; Ramírez-Pinilla et al., 2002; Thompson et al., 2002; Flemming & Blackburn, 2003). In this thesis, therefore, these viviparous reptiles, which demonstrate high levels of matrotrophy, are referred to as "matrotrophic viviparous reptiles".

A large number of studies have investigated why viviparity has evolved in reptiles (e.g. Weekes, 1935; Neill, 1964; Packard, 1966; Greene, 1970; Packard et al., 1977; Shine & Berry, 1978; Shine & Bull, 1979; Shine, 1983, 1995; Qualls & Andrews, 1999; Hodges,

2004), but we lack knowledge about the selective advantages of matrotrophic viviparity in reptiles. For example, we do not know when matrotrophic reproduction is favoured and why it has evolved in relatively few species of reptiles. If the benefits of matrotrophic viviparity in reptiles are related to high maternal energy availability during gestation (see above), the selective advantage of matrotrophic viviparity in reptiles may be not be driven only by maternal thermoregulation during gestation (see the Maternal Manipulation Hypothesis in section 1-1). This is because in many animals, including reptiles, net energy gain is strongly associated with the interaction between many factors such as body temperature, plasma corticosterone concentration and food availability (Spencer et al., 1998; Preest & Cree, 2008; Tsai et al., 2009). However, no study has investigated the relationship between maternal net energy availability during gestation and offspring fitness in matrotrophic viviparous reptiles. As regards pre-adaptations for matrotrophic reproduction, energy recycling (e.g. eating infertile eggs and stillborn offspring) and abortion have been documented in some matrotrophic viviparous reptiles (Blackburn et al., 1998, 2003; Shine & Downes, 1999; Wapstra, 2000). However, there is no information on whether bet-hedging strategies exist in matrotrophic viviparous reptiles.

1-4. Maternal effects

Maternal effects occur when the maternal environment or phenotype affects variation in offspring phenotypes over and above the direct effect of transmitted genes. They commonly occur in both plants and animals and may have a significant influence on evolutionary ecology (Mousseau & Fox, 1998). Although we have accumulated knowledge of maternal effects over nearly 100 years (e.g. Dobzhansky, 1935; Galloway, 2005; Lindholm et al., 2006; Karell et al., 2008; Hodge et al., 2009; Bischoff & Müller-Schärer, 2010; Wapstra et al., 2010), the adaptive significance of maternal effects is still an unresolved issue in evolutionary biology (Mousseau et al., 2009). This is because maternal effects may occur without any important ecological implications (Fox & Czesak, 2000; Einum & Fleming, 2004; Mainwaring et al., 2010) or conversely they may show positive or negative impacts on offspring fitness (Marshall & Uller, 2007). Furthermore, maternal effects may not persist and, therefore, they may not be ecologically relevant to future life-history stages (Bernardo, 1996; Heath & Blouw, 1998; Lindholm et al., 2006; Buckley et al., 2007; Robbins & Warner, 2010).

Recently, Marshall & Uller (2007) and Uller (2008) classified maternal effects into four

types. Firstly, context-dependent or anticipatory maternal effects occur when maternal environment is a reliable predictor of the offspring environment (Marshall & Uller, 2007; Uller, 2008). Mothers adjust offspring phenotype in response to local environmental conditions, and thus offspring fitness is enhanced (e.g. Fox et al., 1997; Kudo & Nakahira, 2005). Secondly, bet-hedging maternal effects (diversified bet-hedging) produce phenotypic variation within-clutch that experiences the same postnatal environment, and thus some, but not all, offspring are likely to show high fitness (Marshall & Uller, 2007). Selection usually favours this strategy when the environmental conditions experienced by offspring are unpredictable from the maternal environmental conditions (Marshall & Uller, 2007; Marshall et al., 2008; Crean & Marshall, 2009). Thirdly, selfish maternal effects occur when the costs of current reproduction outweigh maternal fitness (Marshall & Uller, 2007). They reduce current reproductive investment for future reproduction and therefore enhance maternal fitness but decrease offspring fitness (e.g. Festa-Bianchet & Jorgenson, 1998; Hanssen et al., 2002). Finally, transmissive maternal effects occur when environmental variations affect offspring phenotype through the mothers; therefore, offspring fitness is dependent on what factors are transmitted by the mother (e.g. Bernardo, 1996; Cadby et al., 2010).

1-5. Maternal effects in reptiles

Temperature during embryogenesis is well known to affect offspring phenotype in all vertebrate taxa (Gillooly & Dodson, 2000). In ectotherms such as reptiles, however, the importance and impact of environmental temperature related embryonic development are greater than in mammals. This is because embryonic temperature has a marked effect on embryogenesis in reptiles (Shine & Harlow, 1996; Wapstra, 2000; Goodman, 2008; Wapstra et al., 2009) and, compared with mammals, embryonic temperature during embryogenesis in reptiles is highly dependent on environmental temperature. Maternal thermoregulation in viviparous reptiles and nest choice in oviparous reptiles may control temperature of embryonic development (Roosenburg, 1996; Rock et al., 2002; Warner & Shine, 2007b; Robert & Thompson, 2010), but it is not as perfect as mammals. Thus most studies of maternal effects in reptiles have focused on effects of environmental temperature during embryogenesis on offspring phenotype. Temperature during embryonic development in reptiles may affect offspring sex (Warner et al., 2007; Radder et al., 2008; Wapstra et al., 2009), date of hatching or birth (Warner & Shine, 2007a; Goodman, 2008; Uller & Olsson, 2010; Wapstra et al., 2010), offspring size (Shine &

Harlow, 1996; Wapstra, 2000), offspring performance ability and behaviour (Elphick & Shine, 1998; Ji et al., 2006) and offspring growth rate (Rhen & Lang, 1995; O'Steen, 1998; Wapstra, 2000).

Amongst reptiles, viviparous species extend the duration of maternal influences on embryonic development further than oviparous species because of their longer egg/embryo retention. Extended egg retention increases maternal influence through the maternal-embryonic communications including hormone and nutrient transfer via the placenta. Exposure to maternal hormones such as corticosterone and testosterone during embryogenesis significantly affects embryonic development and consequently offspring phenotype in many animals including viviparous reptiles (Uller et al., 2007; Cucco et al., 2008; Cadby et al., 2010; Harris & Seckl, 2011). For example, maternal corticosterone exposure during gestation in viviparous reptiles may affect offspring size (Meylan & Clobert, 2005; Cadby et al., 2010), offspring performance ability and behaviour (Meylan & Clobert, 2004), offspring body condition (Vercken et al 2007; Cadby et al., 2010), and offspring growth rate (Meylan & Clobert, 2005). Similarly, maternal energy availability during gestation may affect offspring phenotype (Shine & Downes, 1999; Swain & Jones, 2000; Fowden et al., 2006). Notably, viviparous reptiles may invest extra energy in current reproduction to enhance offspring fitness when conditions allow (Swain & Jones, 2000; Jones & Swain, 2006). This extra energy investment has been defined as facultative placentotrophy (Stewart, 1989) because this supplementation is not required for successful development. However, studies into the effects of maternal food availability during gestation in viviparous reptiles are relatively scarce (e.g. Swain & Jones, 2000; Lourdais et al., 2002), despite these effects of maternal food availability may influence offspring size (Shine & Downes, 1999) and offspring body condition (Swain & Jones, 2000).

In matrotrophic viviparous reptiles in particular, the degree and timing of placental inorganic and organic substance (e.g. hormones, inorganic nutrients, organic nutrients and carotenoids) transfer during pregnancy may be significant for embryonic development. This is because there is variation in the sensitive periods when organs and body parts are developing (Jones, 1991; Neaves et al., 2006; Shine et al., 2007). Matrotrophic animals may have a large placental surface area which implies greater possibility of hormone transfer between the mother and the embryos during gestation (Winter et al., 1981; Moore & Lindzey, 1992). Matrotrophic reproduction also reduces yolk volume which implies

reliance on income food as the source of some important organic substances such as carotenoids for embryonic development (Thompson et al., 1999b). Carotenoid availability during embryogenesis may influence embryonic and offspring immune systems and antioxidant capacities (Tachibana et al., 1997; Royle, et al., 2001; Møller & Saino, 2004; Biard et al., 2005). Therefore, maternal plasma hormone concentration and maternal food availability (i.e. quality and quantity) during gestation may strongly affect offspring phenotype in matrotrophic animals (Anderson et al., 1980; Garcia et al., 2003; Fowden et al., 2006; Harris & Seckl, 2011). In matrotrophic viviparous reptiles, however, only a few papers have investigated both the effects of maternal plasma hormone concentration (i.e. corticosterone) during gestation on offspring phenotype (*N. ocellatus*) (Cadby et al., 2010) and the effects of maternal food availability during gestation on offspring phenotype (*P. pagenstecheri*) (Shine & Downes, 1999). Furthermore, no study has investigated the effects of maternal food quality (e.g. carotenoid levels) during gestation on offspring phenotype.

The important point here is that most these offspring phenotypes in viviparous reptiles, including matrotrophic species, result from transmissive maternal effects, although a few studies have also suggested other maternal effects such as context-dependent maternal effects (Shine & Downes, 1999; Meylan & Clobert, 2005). The fitness costs and benefits of transmissive maternal effects depend on environmental conditions during gestation (Marshall & Uller, 2007). If the evolution of viviparity and matrotrophic viviparity in reptiles have been associated with maternal effects, environmental conditions during gestation must have a positive impact on their reproduction more often than not, and maternal physiological mechanisms may exist to maximise the benefits of these maternal effects.

1-6. Research aims

The overall aim of my PhD study is to address the question of why matrotrophic viviparity has evolved in some reptiles. To achieve this, my thesis focuses on two primary objectives: to document placental nutrient and hormone transfer during gestation in a matrotrophic viviparous lizard (*P. entrecasteauxii*) in the first two investigations (Chapters 2 & 3); and to document maternal effects and assess their adaptive significance in a matrotrophic viviparous lizard (*P. entrecasteauxii*) in the last three investigations (Chapters 4, 5 & 6). The first two chapters investigate the major assumptions of the last

three chapters, and also contribute to explanations for the findings of Chapters 4 & 5. The last three chapters will provide possible explanations of selective advantage of matrotrophy in a viviparous reptile. Integration of my investigations (i.e. Chapter 7) will allow me to enhance our understanding of the selective advantage of matrotrophy and physiological mechanisms in *P. entrecasteauxii*. Thus, this study will contribute to an overall understanding of the importance of maternal effects and links between maternal effects and evolution of matrotrophic viviparity in reptiles. A brief description of the aims of each data chapter is below.

Chapter 2:

A previous study showed that the mean dry weight of newly ovulated eggs were much smaller than that of the offspring in *P. entrecasteauxii* (Stewart & Thompson, 1993). This suggests that their embryonic development may be strongly supported by placental nutrient transfer during gestation. I aim to confirm this and to provide direct evidence of this maternal nutrient transfer to support embryonic development during gestation. I will examine the relationship between the degree of placental nutrient supply and embryonic stage during gestation, and its implications. This investigation will underpin the experimental work (i.e. Chapters 4, 5 & 6).

Chapter 3:

We know that transfer of maternal glucocorticoids across the placenta to the embryo does occur in mammals (Zarrow et al., 1970), and does influence offspring phenotype (Kapoor & Matthews, 2005; Igosheva et al., 2007). In viviparous reptiles, based on previous studies (e.g. Meylan & Clobert, 2005; Vercken et al., 2007; Cadby et al., 2010), we assume that maternal corticosterone passes through the placenta into the embryos during gestation. However, it has yet to be examined. I aim to provide direct evidence that circulating maternal plasma corticosterone is transferred into the embryos during embryonic development in a viviparous reptile. I will examine the relationship between the degree of circulating maternal plasma corticosterone transfer into the embryos and embryonic stage during gestation, and its implications. This investigation will underpin the experimental work (i.e. Chapter 5).

Chapters 4 & 5:

Offspring exposed to maternal corticosterone during embryogenesis are known to express many phenotypic changes in physiological function, behaviour, sex, body condition and/or body size (Hayward & Wingfield, 2004; Igosheva et al., 2007; Uller & Olsson, 2006; Cadby et al., 2010). However, maternal plasma corticosterone concentration and maternal body temperature also affect energetic demand (Preest & Cree, 2008). Food availability during gestation varies between spatial and temporal scales (e.g. Bronikowski & Arnold, 1999; Mills et al., 2008). Therefore, net energy availability in animals may depend on the interaction between body temperature, plasma corticosterone concentration and food availability. Energy budget in animals is known to influence reproductive investment and, therefore, offspring fitness. Notably, in matrotrophic species, availability of maternal net energy during gestation may be a key factor for their selective advantage (Trexler & DeAngelis, 2003; Marsh-Matthews & Deaton, 2006; Ostrovsky et al., 2009). Thus, I aim to investigate the links between maternal effects and net energy availability related to environmental factors, including temperature, stress (corticosterone) and food, during gestation in a matrotrophic viviparous reptile. I will examine the effects of maternal basking opportunity, maternal food availability and their interaction during gestation on offspring fitness (Chapter 4) and will investigate effects of maternal plasma corticosterone concentration, maternal food availability and their interaction during gestation on offspring fitness (Chapter 5).

Chapter 6:

Matrotrophic reproduction allows females to reduce the volume of yolk. This implies that some important organic substances for embryonic development may be reliant on income resources during gestation. In matrotrophic viviparous reptiles, the yolk may not contain any carotenoids (Thompson et al., 1999b). If this is so, matrotrophic viviparous reptiles utilise income carotenoids for embryonic development during gestation. This is because carotenoids, especially β-carotene, are known to affect the immune system which is a fundamental trait for fitness in animals (Schmid-Hempel, 2003; Møller & Saino, 2004). Offspring immune capacity at birth may be improved by maternal carotenoid intake during egg formation or gestation in birds and mammals respectively (e.g. Garcia et al., 2003; Haq et al., 1996; Karadas et al.,

2005). To date, no such study has been done on reptiles. I aim to investigate the links between the selective advantage and quality of income food during gestation in a matrotrophic viviparous reptile. I will examine the effects of maternal β-carotene availability during gestation on offspring immune response.

1-7. Study species

For my study, I chose southern grass skinks, *Pseudemoia entrecasteauxii*. *Pseudemoia* spp. are excellent model species for research into the evolutionary transition from predominantly lecithotrophic viviparous reptiles to highly matrotrophic viviparous reptiles (e.g. some *Mabuya* spp.) because they are moderately matrotrophic (i.e. demonstrate about 20-50 % of matrotrophic support) (reviewed in Thompson et al., 2002). Furthermore, placental structure, placental function, placental ontogeny and other physiological mechanisms during gestation in *Pseudemoia* spp., including *P. entrecasteauxii*, have been well studied (e.g. Stewart & Thompson, 1993, 1996, 1998, 2000; Thompson et al., 1999a, 1999b, 2006; Blackburn et al., 2003; Speake et al., 2004; Adams et al., 2005; Stewart et al., 2006; Thompson & Speake, 2006; Biazik et al., 2009).

Pseudemoia entrecasteauxii is one of the few known species of matrotrophic viviparous reptiles with Type III placentae. Their nutrient provisioning for embryonic development is roughly half from the yolk and half via the placenta (Stewart & Thompson, 1993). Placental ontogeny during gestation including that of placentome in *P. entrecasteauxii* has been well described (Stewart & Thompson, 1996): at embryonic stage 30 of the 0 - 40 (40 is immediately pre-birth) classification scale defined by Dufaure & Hubert (1961), the omphaloplacenta and the chorioallantoic placenta including the placentome begin to develop. By embryonic stage 34, the placentae are richly vascularized and retain highly vascular systems to the end of gestation. Pregnant *P. entrecasteauxii* increase basking behaviour (Shine, 1980) to maintain body temperature for optimum embryonic development. They do not reduce feeding rates during gestation as do other viviparous reptiles (Shine, 1980; Gregory et al., 1999). This feeding behaviour allows them to accumulate energy for placental nutrient support. In addition, they are opportunistic feeders and have a varied diet including invertebrate animals such as insects, plants and fungi (Brown, 1988). Thus, we know the placental ontogeny, and the degree of placental support and feeding behaviour suggest the importance of energy gain during gestation in *P. entrecasteauxii*, making this an appropriate choice of study species to address the aims of my PhD.

Pseudemoia entrecasteauxii is small ground-dwelling lizards [females rarely exceed 60 mm snout-vent length (SVL), males rarely exceed 50 mm] (Hutchinson et al., 2001). Their distribution is confined to the south-eastern Australian mainland, Tasmania and the Bass Strait Islands (Wilson & Swan, 2008). In Tasmania, populations of *P. entrecasteauxii* are large, widely distributed, and occupy sites with a variety of habitats from sea level to about 1000 m in low to tall open forest, woodland, heathland, and alpine herbfields (Wilson & Knowles, 1988; Cogger, 1992). Their reproductive cycle has been described as autumn spermatogenesis and mating, spring vitellogenesis and ovulation, and the young are born in early January to late February (Heatwole & Taylor, 1987; Murphy et al., 2006).

1-8. Study sites

All samples in this study were collected from Tasmania, Australia. Samples for Chapter 2 were collected during summer 2007 at Pulchella Nursery (open grassland) (42˚36'S, 147˚40'E; altitude 198 m) (Fig. 1.1). Samples for Chapter 3 were collected during summer 2008 at a locality in southern Tasmania (dry sclerophyll forest) (43˚02' S, 147˚15' E; altitude 38 m) (Fig. 1.1). Samples for Chapter 4, Chapter 5 and Chapter 6 were collected during summer 2007 (Chapter 4) and during summer 2008 (Chapters 5 & 6) at the Peter Murrell Reserve in Kingston, southern Tasmania (dry sclerophyll forest) (41˚50'S, 146˚36'E; altitude 116 m) (Fig. 1.1). Samples for Chapter 5 (i.e. examination of plasma CORT concentration) and Chapter 6 (i.e. examination of carotenoid content in the eggs) were collected during spring 2008 at the University of Tasmania (open grassland) (42˚54'S, 147˚19'E; altitude 249 m) (Fig. 1.1).

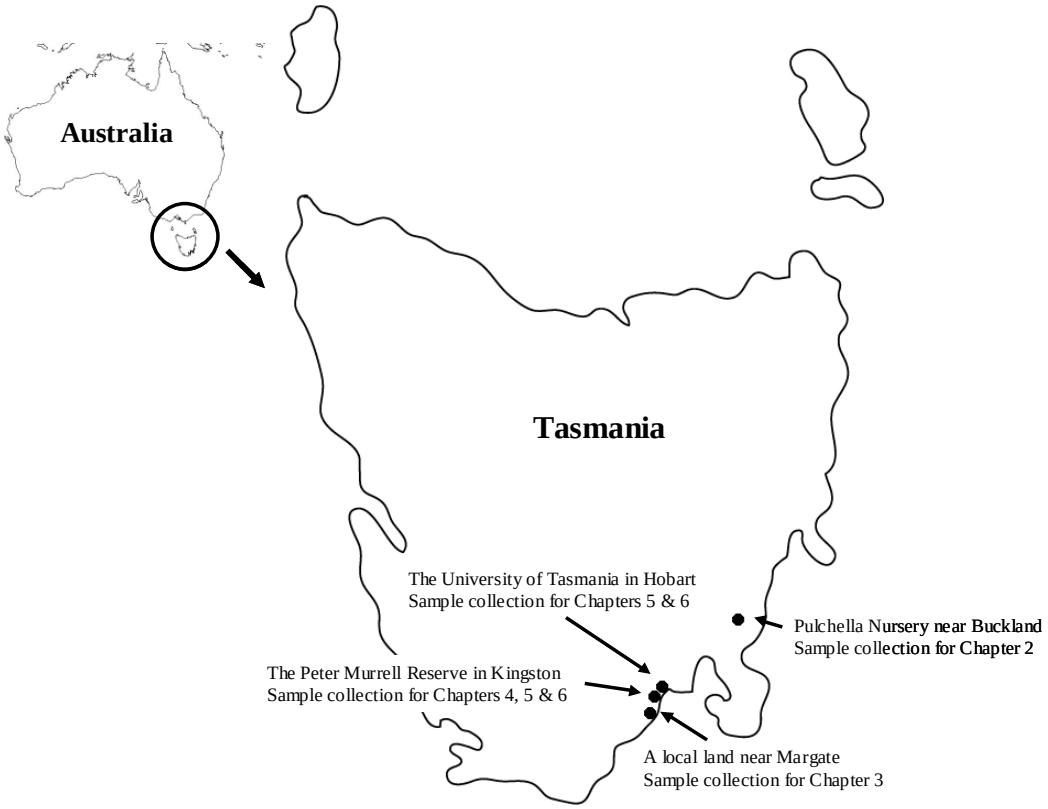

Fig. 1.1. A map of sample locations for my PhD study in Tasmania, Australia.

1-9. Presentation of the thesis

This thesis comprises five data chapters divided into two specific areas associated with the key research objectives described above. All experimental chapters are written as stand-alone scientific papers that have been submitted for publication. I am the primary author on all manuscripts, having undertaken the data collection, data analysis, and preparation of manuscripts; however, I have recognised the contributions of others by acknowledging them as co-authors. Publication status and authorship of individual manuscripts varies, and details are provided at "Statement of Co-Authorship (page ii)" or at the beginning of each chapter.

By necessity, each experimental chapter may involve some repetition, particularly in terms of animal collection and species descriptions. In addition, abstracts are included within each chapter, with the thesis abstract providing a broader summary of the thesis. Formatting between chapters is not necessarily uniform because of the requirements of different format and style of each of journals. The content of each manuscript remains as submitted for publication where relevant.

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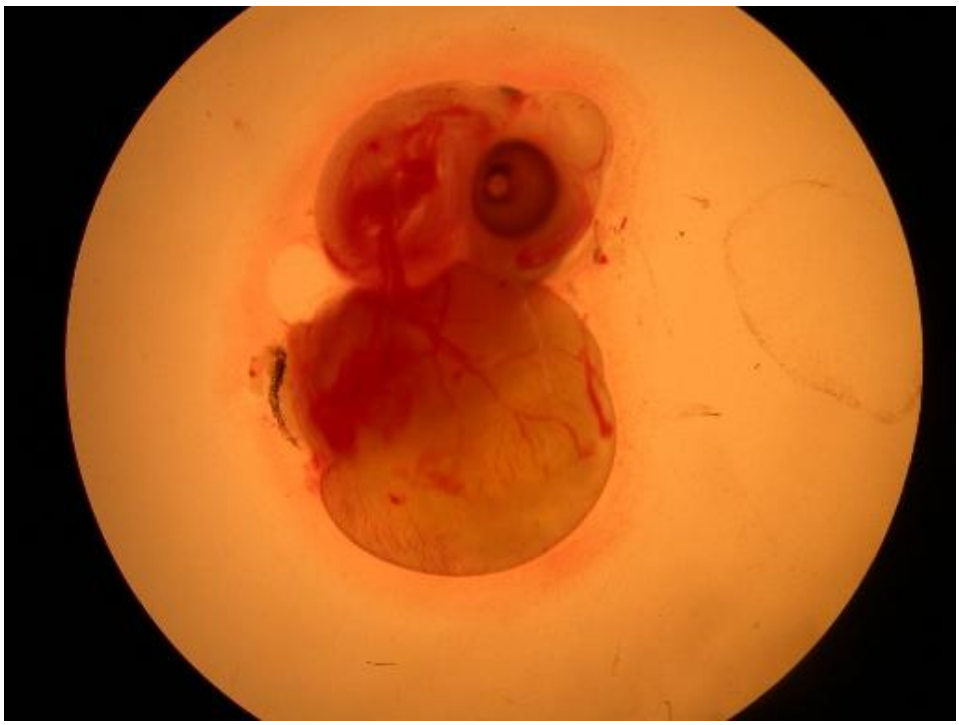
Section I.

Physiological mechanisms during gestation in *Pseudemoia entrecasteauxii*



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A number of hypotheses for the evolution of viviparity in reptiles have been proposed since the early twentieth century (Weekes, 1935; Neill, 1964; Packard, 1966; Shine & Bull, 1979; Tinkle & Gibbons, 1977; Shine, 1995). Currently, there is one generally accepted hypothesis, the Maternal Manipulation Hypothesis (Shine, 1995). This hypothesis focuses on the relationship between egg retention and offspring fitness benefits through maternal effects such as maternal thermoregulation (Shine, 1995). However, the importance of maternal effects is still the subject of ongoing debate. In squamate reptiles, viviparity has evolved from about 100 independent lineages, and matrotrophic viviparity has evolved from at least four of these lineages (Shine, 1985; Blackburn, 1992, 2000, 2006; Flemming & Branch, 2001). These multiple origins suggest a potential multitude of selective pressures and selective advantages. Therefore, it is possible that there are multiple explanations (e.g. Weekes, 1935; Neill, 1964; Packard, 1966; Shine & Bull, 1979; Tinkle & Gibbons, 1977; Shine, 1995) for the evolution of viviparous reproduction, including matrotrophic reproduction in reptiles. Similarly, the evolution of matrotrophic viviparity in fish may have several explanations given its multiple origins (Blackburn et al., 1985; Trexler & DeAngelis, 2003; Marsh-Matthews & Deaton, 2006; Banet & Reznick, 2008; Banet et al., 2010). Research into the evolution of viviparity and matrotrophy using viviparous animals with multiple origins, especially those with a large number of independent origins like reptiles and fish (Blackburn, 1992), will provide a broader view of the evolution of viviparity and matrotrophy in animals.

7-1. Evolution of matrotrophy in Pseudemoia entrecasteauxii

The trade-offs between fitness costs and benefits related to maternal energy availability during gestation may have been an important determinant for the evolution of matrotrophic reproduction in *P. entrecasteauxii* (Chapter 4). Matrotrophic reproduction may have a selective advantage when maternal energy availability is high during gestation (Trexler & DeAngelis, 2003; Marsh-Matthews & Deaton, 2006; Ostrovsky et al., 2009). When maternal energy availability is high during gestation, female *P. entrecasteauxii* produce offspring with early birth date, large body size, large fat reserves relative to body mass and fast sprint speed (Chapter 4). In *P. entrecasteauxii*, the degree of placental nutrient transfer during gestation significantly influences somatic growth of the embryos (Chapter 2). High maternal nutrient transfer during gestation may enhance offspring size and fat reserves (Chapter 2; Swain & Jones, 1997; Jones & Swain, 2006). Therefore, high maternal energy availability during gestation may allow females to invest

large amounts of energy in current reproduction. Further, these offspring phenotypic traits, including early birth date, large body size, large fat reserves relative to body mass and fast sprint speed, may enhance offspring fitness in reptiles including *P. entrecasteauxii* (Chapter 4; Janzen et al., 2000; Brown & Shine, 2005; Kissner & Weatherhead, 2005; Burgess et al., 2006; Atkins et al., 2007; Warner & Shine, 2007, While et al., 2009; Uller & Olsson, 2010). Thus, in *P. entrecasteauxii*, high maternal energy availability during gestation may confer significant fitness benefits, and the advantage of matrotrophy is an adjustment of inputs to offspring when conditions allow.

In contrast, when maternal energy availability is low during gestation, female *P. entrecasteauxii* produce variation in offspring phenotype within-clutch, demonstrating a bet-hedging strategy (i.e. variation in body size within-clutch and/or the mixture of full-developed and premature offspring within-clutch) (Chapter 4). This strategy allows females to enhance some offspring fitness within a single clutch, at the sacrifice of fitness of other siblings, and to reduce the costs on offspring fitness of matrotrophy (Chapter 4; Kozlowski & Stearns, 1989; Gaillard & Yoccoz, 2003). Furthermore, females that had low food availability during gestation showed lower postpartum body mass than females that had high food availability during gestation (Chapter 4). This suggests that gravid *P. entrecasteauxii* may use their own energy reserves to maintain reproduction when food is limited during gestation (e.g. Banet & Reznick, 2008; Banet et al., 2010). At present, we do not know the impacts of the reduction of maternal body condition on maternal survival in this species. It is possible that these females reduce the probability of survival for the next breeding season (e.g. Bonnet et al., 1999). However, postpartum *P. entrecasteauxii* increase their activity and have several months before the following winter (Itonaga, 2005). Therefore, female *P. entrecasteauxii* may accumulate enough energy for survival after parturition (e.g. Bonnet et al., 2002), even though they reduce their own body condition for reproduction.

The key evolutionary question here is how often *P. entrecasteauxii* obtains fitness benefits (i.e. high energy availability during gestation) from matrotrophy. A simple answer is "more often than not" because matrotrophic reproduction has been selected. In *P. entrecasteauxii*, vitellogenesis occurs during early spring when food availability may be relatively low and unpredictable compared with the gestational periods (i.e. late-spring to summer) (Heatwole & Taylor, 1987; Murphy et al., 2006). High temperatures are more

frequently experienced during gestation than during vitellogenesis. Thus, the interaction between high food availability and high temperature during gestation may enhance net energy availability (Tsai et al., 2009). In addition, the long daily activity time during gestation, compared with during vitellogenesis, may increase daily energy assimilation (Adolph & Porter, 1993; Angilletta, 2001). In nature, therefore, gravid *P. entrecasteauxii* may obtain more net energy per day during gestation than during vitellogenesis. Supporting this suggestion, in natural populations of another matrotrophic viviparous reptile (*Niveoscincus ocellatus*), which inhabit the same region as *P. entrecasteauxii*, gravid *N. ocellatus* accumulate their own fat reserves during gestation, even though they allocate energy to embryonic development via the placentae (Thompson et al., 2001; Wapstra & Swain, 2001). This finding implies high net energy availability during gestation (late-spring to summer) in nature. Gravid *P. entrecasteauxii* can also enhance net energy gain during gestation by increasing basking frequency and maintaining high feeding rates (Shine 1980; Spencer et al. 1998; Tsai et al. 2009), although such behaviours can increase predation risks, especially for gravid females (Shine, 1980; Huey & Pianka 1981; Abrams, 1991; Lima, 1998). These behaviours and their relation to the risks suggest that the benefits of energy gain during gestation may outweigh the costs. Thus, predictably high energy availability during gestation may lead to selection for placental nutrient supply (i.e. producing offspring with high fitness) and thus the evolution of matrotrophy in *P. entrecasteauxii*. I call this idea the "Maternal Energy Hypothesis".

7-2. A major challenge: how does the maternal energy hypothesis fit for other reptiles?

Among the currently known matrotrophic viviparous reptiles, *Chalcides chalcides*, *N. ocellatus*, *P. entrecasteauxii*, *P. pagenstecheri* and *P. spenceri* are at least moderately matrotrophic (i.e. reduction in about 20-80 % of yolk volume), whereas *Mabuya* spp. in the New World (i.e. *M. bistriata, M. heathi, M. mabouya*) and *Eumecia anchietae* are extremely matrotrophic (i.e. reduction in more than 99 % of yolk volume) (Ghiara et al., 1987; Ramírez-Pinilla et al., 2002; Thompson et al., 2002; Flemming & Blackburn, 2003). In addition to these species, many other *Mabuya* spp. in the New World (e.g. *M. caissara*, *M. frenata*, *M. macrorhyncha* and *M. unimarginata*) are regarded as extremely matrotrophic, although the degree of placental nutrient transfer has not been examined (e.g. Fitch, 1970; Vanzolini & Reboucas-Spieker, 1976; Blackburn & Vitt, 1992; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999). If the maternal energy hypothesis applies to the evolution of matrotrophic viviparity in reptiles like *P. entrecasteauxii*

(Chapter 4 and previous section), high energy availability during gestation is a key selective agent. So far, only a single paper has investigated effects of food quantity during gestation in a matrotrophic viviparous reptile (*P. pagenstecheri*) (Shine & Downes, 1999), and their study, which found that high maternal food availability during gestation produces large offspring, may support the maternal energy hypothesis. However, it is difficult to conclude that the maternal energy hypothesis is the general explanation for the evolution of matrotrophic viviparity in reptiles because of the shortage of empirical evidence, although food availability during gestation affects offspring fitness in other matrotrophic animals such as mammals (Anderson et al., 1980; Fowden et al., 2006; Harris & Seckl, 2010).

The major assumptions of the Maternal Energy Hypothesis are predictably high energy availability during gestation and some counter strategies to the costs of matrotrophy, such as energy recycling, abortion and bet-hedging (Kozlowski & Stearns, 1989; Greeff et al., 1999; Trexler & DeAngelis, 2003; reviewed in Gaillard & Yoccoz, 2003). All currently known species of moderately matrotrophic viviparous reptiles inhabit temperate regions (Hutchinson et al., 2001; Wilson & Swan, 2008; cf. Guarino, 2010), and they demonstrate a similar reproductive cycle: vitellogenesis takes place in autumn and/or early spring, ovulation in spring, and gestation in summer (Heatwole & Taylor, 1987; Jones et al., 1997; Thompson et al., 1999a, 1999b; Paulesu et al., 2001; Murphy et al., 2006). In this reproductive cycle, animals may experience high energy availability during gestation (e.g. Wapstra & Swain, 2001) because of high temperature, high food abundance and long daily activity time (Heatwole & Taylor, 1987; Adolph & Porter, 1993; Angilletta, 2001; see previous section). Similarly in some possible counter strategies to the costs on matrotrophy, recycling of energy (e.g. eating infertile eggs and stillborn offspring) and abortion has been documented in most moderately matrotrophic viviparous reptiles (e.g. Blackburn et al., 1998, 2003; Shine & Downes, 1999; Wapstra, 2000), although there is no information about bet-hedging in all the moderately matrotrophic viviparous reptiles except *P. entrecasteauxii* (Chapter 4). Therefore, if high maternal energy availability during gestation has positive effects on overall fitness (Trexler & DeAngelis, 2003), at least for the moderately matrotrophic viviparous reptiles, the maternal energy hypothesis may apply to the evolution of matrotrophic reproduction. If this suggestion is right, many other viviparous reptiles which demonstrate similar reproductive cycle to the moderately matrotrophic viviparous reptiles living in temperate regions (e.g. Heatwole & Taylor,

1987; Jones & Swain, 1996) also experience high energy availability during gestation. In temperate regions, however, a large number of viviparous reptiles are predominantly lecithotrophic and a number of matrotrophic viviparous reptiles is very small. I will discuss possible explanations for this relative rarity in the last part of this section.

Many extremely matrotrophic viviparous reptiles such as *Mabuya* spp. in the New World (e.g. *M. bistriata* and *M. heathi*) inhabit tropical regions (wet-dry seasonally), and their vitellogenesis and ovulation occur during the wet season when food is abundant, and rapid embryonic development occurs during the dry season when food is scarce (i.e. the last 5-6 months of gestation in total 9-12 month gestational periods) (Janzen & Schoener, 1968; Vitt & Blackburn, 1983, 1991). Evidence from these studies may not support the idea of the maternal energy hypothesis, which suggests that a different selective advantage is associated with extremely matrotrophic viviparous reptiles. For example, many *Mabuya* spp. in the New World (e.g. *M. bistriata, M. frenata, M. heathi* and *M. mabouya*) produce very tiny yolks, and this allows them to reproduce at young ages (i.e. several months old) with small body size (i.e. juvenile body size) (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998; Ramírez-Pinilla et al., 2002). Their gestation begins during the wet season, which allows these small females to undergo rapid growth and to reach adult body size before rapid embryonic development occurs (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998; Ramírez-Pinilla et al., 2002). Evolutionary life-history theory predicts that the strategy of reproducing at an early age is associated with high mortality (Adolph & Porter, 1993; Charlesworth, 1994; Kraus et al., 2005). Therefore, the evolution of extreme matrotrophic viviparity in reptiles (i.e. facilitating early reproduction) may have been driven by environments with high mortality in the tropics (Adolph & Porter, 1993). Similarly, high mortality-induced matrotrophic reproduction has been suggested in a matrotrophic viviparous fish (Banet & Reznick, 2008).

Differences in reproductive strategy between moderately and extremely matrotrophic viviparous reptiles are age at maturity, volume of yolk and duration of gestation. The former take more time to be mature (2-4 years v.s. generally a few months), produce more yolk and have much shorter gestational periods (about 3-4 months v.s. 9-12 months) than the latter (Vitt & Blackburn, 1983, 1991; Ghiara et al., 1987; Rocha & Vrcibradic, 1999; Wapstra et al., 2001; Ramírez-Pinilla et al., 2002; Thompson et al., 2002; Murphy et al.,

2006; Guarino, 2010; cf. Stapley, 2008). Moderately matrotrophic reproduction may be suitable for temperate regions. This is because the temperate climates restrict annual activity time including gestation (Fitch, 1970; Adolph & Porter, 1993; Wapstra et al., 1999; Edwards et al., 2002; Guarino, 2010). Short gestational periods and/or the energy use by gravid females for enhancing their own survival and/or future reproduction (e.g. Chapter 4; Wapstra & Swain, 2001) may restrict the degree of placental nutrient transfer for embryonic development. Therefore, the production of some yolk may be important to support embryonic development in temperate climates. This yolky support allows moderately matrotrophic viviparous reptiles to reproduce annually like many other oviparous reptiles in temperate climates (Fitch, 1970; Heatwole & Taylor, 1987; Wapstra et al., 1999; Murphy et al., 2006). In contrast, extreme matrotrophic reproduction in viviparous reptiles may facilitate early reproduction. The production of very tiny yolks and rapid growth in gravid females, especially small females, during first 4-6 months of gestation (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999; Ramírez-Pinilla et al., 2002) implies that they need large amounts of income energy for both reproduction and growth and time for maternal growth. Tropical climates allow extremely matrotrophic viviparous reptiles to be active all year and, therefore, they extend gestational periods and reproduce annually (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999; Ramírez-Pinilla et al., 2002).

Matrotrophic reproduction in viviparous reptiles has fitness benefits and it is possible that variation in life history traits and selection shape the degree of matrotrophy in viviparous reptiles through different selective advantages (e.g. Chapter 4; Vitt & Blackburn, 1983). However, the majority of reptiles are oviparous: viviparity is less common and matrotrophic viviparity is rare. A possible reason for this relative rarity is that viviparous reproduction may be costly because annual reproductive frequency and consequently fecundity are usually higher in oviparous reptiles than in viviparous reptiles (Fitch, 1970; Tinkle & Gibbons, 1977; Vitt & Blackburn, 1983, 1991; Vitt, 1986; Heatwole & Taylor, 1987; Adolph & Porter, 1993; Hutchinson et al., 2001; Ramírez-Sandoval et al., 2006). Lecithotrophic reproduction also favours unpredictable environments (i.e. food levels) during embryogenesis (Thibault & Schultz, 1978; Wourms & Lombardi, 1992). In addition, pregnancy is usually associated with an increase in basking frequency and reduction of performance ability, which can increase the risk of predation (Shine, 1980; Van Damme et al., 1989; Sinervo et al., 1991; Wapstra & O'Reilly, 2001; Robert &

Thompson, 2010). Thus, viviparity in reptiles evolves when the benefits [the Cold Climate Hypothesis (Tinkle & Gibbons, 1977; Shine, 1983) or the Maternal Manipulation Hypothesis (Shine, 1995) see earlier] outweigh these costs. In addition, matrotrophic viviparity in reptiles potentially increases the costs of viviparous reproduction. For example, matrotrophic viviparous reproduction may increase the risk of predation because this reproduction requires relatively high maternal feeding rates during gestation for placental nutrient support and an increase in foraging activity may enhance predation risks (Shine, 1980; Huey & Pianka, 1981; Abrams, 1991; Lima, 1998). Indeed, many predominantly lecithotrophic viviparous reptiles reduce feeding during gestation (Shine, 1980; Gregory et al., 1999). For many species, therefore, matrotrophic viviparity may not provide a net advantage in terms of fitness. The other possible explanation may be physiological limitations on placental nutrient transfer (i.e. link to placental structure) due to phylogenetic constraints (Stewart & Thompson, 2003). In reptiles, viviparity has about 100 independent origins (Blackburn, 1992) with variation in placental structure (Blackburn, 1992; Stewart & Thompson, 2000; Blackburn & Vitt, 2002). Therefore, some evolutionary origins of placental structure may not have the potential for further evolution of matrotrophic reproduction.

7-3. Consequence of evolution of matrotrophic viviparity in reptiles

As matrotrophic viviparity has evolved in reptiles, some important considerations have arisen. In matrotrophic viviparous reptiles, the volume of yolk is reduced and its composition and/or its volume of composition consequently are changed For example, the yolk may not contain enough carotenoids for successful embryonic development (Chapter 6, Thompson et al., 1999b). This means that development of the embryonic and offspring immune systems, offspring immune capacity, embryonic and offspring antioxidant capacities and offspring pigmentation during embryogenesis or at birth may depend on maternal foraging (i.e. carotenoid intake) during gestation (Chapter 6; Royle, et al., 2001; Surai et al., 2001; Garcia et al., 2003; Koutsos et al., 2003; Biard et al., 2005; Olson & Owens, 2005; Fitze et al., 2007). Notably, immunostimulating and antioxidant effects of carotenoids significantly affect offspring fitness (Tachibana et al., 1997; Royle, et al., 2001; Møller & Saino, 2004; Biard et al., 2005). Relying on income food as the sources of carotenoids for reproduction may be advantageous for matrotrophic viviparous reptiles living in temperate climates because of high food (including carotenoids) availability during gestation (see previous two sections). Conversely, in some matrotrophic viviparous

reptiles (e.g. *M. agilis*, *M. bistriata*, *M. frenata*, *M. heathi* and *M. macrorhyncha*) living in tropical climates (wet-dry seasonally), major embryonic development occurs during the low food dry season (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999). It is not known how these species cope with carotenoid intake during the dry season. Their long gestational periods (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999) may offset potentially low carotenoid availability during the dry season or they use stored carotenoids. Alternatively, they may prefer to feed on carotenoid-rich food during the dry season. There is evidence of shifting food preference in response to reproduction in other taxa. For example, tufted puffins (*Fratercula cirrhata*) take more carotenoid-rich prey before egg formation (Williams et al., 2008; Davies et al., 2009).

Another important consideration is the effects of maternal hormone transfer during gestation on offspring phenotype. Matrotrophic *P. entrecasteauxii* may adjust offspring phenotype in response to maternal corticosterone signals during gestation (i.e. context-dependent maternal effects which produce small offspring body size with large fat reserves in the stressful postnatal environments) (Chapters 3 & 5). Corticosterone alters energy allocation among physiological processes (Frigerio et al., 2001) and, therefore, variation in maternal plasma corticosterone concentration in matrotrophic viviparous reptiles may affect the degree of placental transfer of different nutrients and consequently offspring phenotype. Indeed, the production of small offspring size with large fat reserves in response to maternal plasma corticosterone concentration (Chapter 5) can be explained by a change in the degree of both fatty and amino acid transfer via the placenta during gestation (Chapter 2; Jones & Swain, 2006). Furthermore, offspring growth rate in *P. entrecasteauxii* may respond to postnatal stress levels by maternal corticosterone induced-programming during embryogenesis (Chapters 3 & 5). In a similar experiment on another matrotrophic viviparous reptile (*N. ocellatus*), there was no evidence that such maternal corticosterone signals during gestation induced programming effects on offspring growth rate (Cadby et al., 2010). Therefore, corticosterone programming of the embryos in *P. entrecasteauxii* may be a species-specific reaction to maternal plasma corticosterone concentration during gestation. Compared with mammalian species (e.g. Montano et al., 1993; Burton & Waddell, 1999; Seckl, 2001, 2004; Fowden & Forhead, 2004; Fowden et al., 2005, 2006; Harris & Seckl, 2011), studies of corticosterone programming of embryos (e.g. Chapter 5; Cadby et al., 2010)

and the relationship between maternal corticosterone transfer and its relationship to physiological mechanisms during gestation (e.g. Chapter 3) in matrotrophic viviparous reptiles are very limited, although these reptiles may have large possibility of hormone transfer between the mother and the embryos during gestation (Winter et al., 1981; Moore & Lindzey, 1992).

7-4. Future direction

I suggest one hypothesis (i.e. the Maternal Energy Hypothesis) for the evolution of matrotrophic viviparity in reptiles, although this hypothesis may only apply to matrotrophic viviparous reptiles living in temperate regions (i.e. the moderately matrotrophic viviparous reptiles). This thesis assumes offspring fitness benefits both from their phenotype at birth and from their growth rates observed in relatively benign laboratory conditions. However, this seems plausible as many studies have demonstrated the links between offspring phenotype at birth and fitness in natural population in reptiles (e.g. Janzen et al., 2000; Brown & Shine, 2005; Warner & Shine, 2007; While et al., 2009; Pen et al., 2010; Uller & Olsson, 2010). However, it is still important to investigate the relationship between selection processes in natural populations and offspring phenotype in *P. entrecasteauxii* (i.e. long-term monitoring: at least of offspring survival to first reproduction) to confirm selective advantage though high maternal energy availability during gestation. Furthermore, I suggest applying the same framework for understanding other matrotrophic viviparous reptiles, especially for moderately matrotrophic viviparous reptiles (e.g. other *Pseudemoia* spp. and *N. ocellatus*) to test the maternal energy hypothesis. We also need to investigate what class of nutrients across the placenta of matrotrophic viviparous reptiles. In addition, it may be useful to take climatic or regional differentiation into account when conducting studies into the selective advantage of matrotrophic viviparity in reptiles. One broader view of the evolution of viviparity, especially matrotrophic viviparity in vertebrate animals (i.e. the parent-offspring conflict theory) (reviewed in Crespi & Semeniuk, 2004) is strongly related to the key concept of my hypothesis (i.e. the degree of maternal energy investment). Further studies like this thesis may contribute to our understanding of the evolution of matrotrophic viviparity in reptiles and the links to climate, and also the evolution of viviparity in vertebrate animals.

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